J. New York Entomol. Soc. 109(2):304–308, 2001

MITOCHONDRIAL CYTOCHROME OXIDASE I DNA SEQUENCES AND THE PHYLOGENY OF COCCINELLIDAE (INSECTA: COLEOPTERA: CUCUJOIDEA)

JONATHAN R. MAWDSLEY

Department of Entomology, MRC 187, National Museum of Natural History,
Smithsonian Institution, Washington, D.C. 20560l-0187

Abstract.—A recently-published phylogenetic tree, constructed using the neighbor-joining algorithm, summarized relationships among 37 species of Coleoptera (Insecta) indicated by a 400 base pair region of the mitochondrial cytochrome oxidase I gene. This study included sequences from four species of the family Coccinellidae. The relationships of the four coccinellids indicated by the published neighbor-joining tree are congruent with current hypotheses of their relationships based on adult, larval, and pupal morphology. However, re-analysis of the molecular sequences for these taxa using standard parsimony methods reveals a more complex situation in which the use of different outgroups and different tree-searching algorithms yields strikingly different topologies, many of which do not correspond to the pattern of relationships derived from morphological data. These sequences may simply be too variable and too highly convergent to accurately reflect the phylogenetic history of Coccinellidae.

Howland and Hewitt (1995) published the results of a molecular phylogenetic study that attempted to resolve relationships of higher-level taxa in the order Coleoptera. The topology reported by these authors was based on a neighbor-joining analysis of a 400 base pair region from the mitochondrial cytochrome oxidase I gene. This region was sequenced for 37 species of Coleoptera, representing just two of the four major suborders of Coleoptera, and only 15 of the 166 families currently recognized in this order (Lawrence and Newton 1995).

Howland and Hewitt (1995) noted that their neighbor-joining tree could be rerooted so that species of Coleoptera included in their analysis are divided into two monophyletic groups corresponding to the morphologically-defined suborders Adephaga and Polyphaga. However, these authors were unable to find a real outgroup taxon that would actually root this tree so that both Adephaga and Polyphaga are monophyletic. Furthermore, the relative positions of many of the species in the suborder Polyphaga in the published neighbor-joining tree are highly incongruous with the standard classification of these species based on adult and larval morphology. To their credit, Howland and Hewitt (1995) noted these incongruities, but commented that the relative positions of genera in certain lineages of Polyphaga, including four species belonging to four genera in the family Coccinellidae, nevertheless reflected current classifications.

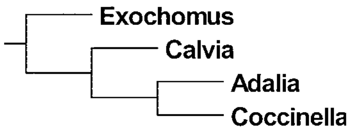
In the present paper, I report the results of a series of re-analyses of the sequences of the four species of Coccinellidae that were included in Howland and Hewitt's original analysis: *Adalia bipunctata* (L.); *Calvia quatuordecimguttata* (L.); *Coccinella novemnotata* Herbst; and *Exochomus* sp. The results presented here clearly demonstrate that the topology reported by Howland and Hewitt for these four coccinellid species is not robust with respect to the selection of different outgroups or to the selection of different tree-searching algorithms.

METHODS OF ANALYSIS

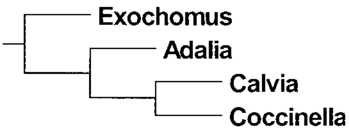
Sequences used in the re-analyses described below were downloaded from GenBank (accession numbers and original sequence alignments, which were also used in the present re-analyses, were published in Howland and Hewitt 1995). These sequences were analyzed using the standard parsimony computer programs Hennig86 (Farris 1988), NONA (Goloboff 1993b) and PIWE (Goloboff 1993a). Individual base pairs in these sequences were analyzed as unordered multistate characters. With Hennig86, sequences were analyzed using the mh* tree-search algorithm and the bb* branch-swapping algorithm. A successive approximations routine was also implemented in Hennig86 using Farris's rescaled consistency index (character retention index times character consistency index) as described by Farris (1988). With NONA, analyses were conducted using twenty replicates of the ‘‘mult’’ tree-searching command followed by tree bisection and reconnection branch-swapping on all trees up to ten steps longer than the most parsimonious tree(s) found during the mult runs. PIWE analyses were conducted using a search routine identical to that described for NONA. Most parsimonious cladograms resulting from these analyses were saved and examined using the WINCLADA package (Nixon 1999).

MORPHOLOGICAL HYPOTHESES OF COCCINELLID RELATIONSHIPS

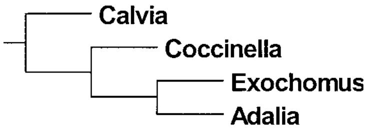
Howland and Hewitt (1995) noted that the pattern of relationships among coccinellid species expressed by their neighbor-joining tree reflected the current classification of these beetles. Specifically, these authors noted that their neighbor-joining tree separated the three species belonging to the subfamily Coccinellinae (*Adalia bipunctata*, *Calvia quatuordecimguttata*, and *Coccinella novemnotata*), from *Exochomus* sp., which belongs in the subfamily Chilicorinae. The classification of Coccinellidae to which Howland and Hewitt refer is the result of nearly two centuries of detailed anatomical examinations of these beetles. Adults and larvae of many species of Coccinellidae are both abundant and economically important, and consequently adult and larval morphological character systems in this family have been extensively studied. In addition to the copious literature on adults and larvae, Phuoc and Stehr (1974) have provided a valuable review of the pupal morphology of Coccinellidae, which offers many phylogenetically informative character systems. Although a comprehensive review of all morphological evidence for the phylogenetic relationships of coccinellids is outside the scope of this paper, I did undertake a survey of standard works on the anatomy and classification of coccinellid beetles, as listed by Lawrence and Newton (1995). Specific works consulted included Fürsch (1995), Gordon (1985), Kamiya (1965), Klausnitzer (1970), Kovář (1996), Phuoc and Stehr (1974), and Sasaji (1968). Taken together, the morphological studies of these authors provide strong support for the following two hypotheses of relationships, which are of direct relevance to the present paper: first, the subfamilies Coccinellinae and Chilicorinae are distinct lineages, each defined by derived character states (whether these two lineages are ranked as subfamilies or tribes is not relevant to the present paper); and second, the genera *Adalia* and *Coccinella* are more closely



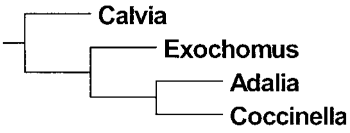
1



2



3



4

Figs. 1–4. Hypotheses of relationships among four species of Coccinellidae, as determined by parsimony analysis of mitochondrial cytochrome oxidase I DNA sequences as described in text. The topology in Fig. 1 is congruent with hypotheses of relationships for these beetles based on adult, larval, and pupal morphology.

related to each other than either of these two genera is to the genus *Calvia.* Therefore, the expected pattern of phylogenetic relationships among the four coccinellid taxa in Howland and Hewitt's data set is: (*Exochomus* (*Calvia* (*Adalia Coccinella*))).

DESCRIPTION AND RESULTS OF RE-ANALYSES

I initially downloaded only those sequences pertaining to the four species of Coccinellidae. Selecting *Exochomus* sp. as outgroup, the two topologies recovered by Hennig86 (using successive weighting and equal weights) and NONA were: (*Exochomus* (*Calvia* (*Adalia Coccinella*))) and (*Exochomus* (*Adalia* (*Calvia Coccinella*))) (Figs. 1–2). PIWE only reported the first of these two topologies. The first topology is congruent with published hypotheses of relationships for these taxa based on adult, larval, and pupal data; the second topology is incongruent with these hypotheses.

One other species in the superfamily Cucujoidea (to which the family Coccinellidae belongs) was included in Howland and Hewitt's data set: *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). Using this species as an outgroup for the four coccinellids, Hennig86 (using both successive weighting and equal weights), NONA, and PIWE recovered the following topology: (*Oryzaephilus* (*Calvia* (*Coccinella* (*Exochomus Adalia*)))) (Fig. 3). This topology places *Exochomus* well within the coccinelline clade, strongly contradicting hypotheses of relationships based on adult, larval, and pupal morphology.

I next decided to employ an outgroup from Howland and Hewitt's data set that is generally considered to be only distantly related to the members of the superfamily Cucujoidea. Accordingly, a sequence representing an unidentified species of the genus *Cercyon* in the family Hydrophilidae (Coleoptera: Polyphaga: Hydrophiloidea) was downloaded and added to the coccinellid matrix. Hennig86 (using both successive weighting and equal weights), NONA, and PIWE all found the same single most parsimonious topology for these five terminals: (*Cercyon* (*Calvia* (*Exochomus* (*Adalia Coccinella*)))) (Fig. 4). This topology also places *Exochomus* within the coccinelline clade, but differs from the topology found in the preceding analysis in recognizing the adelphotaxon relationship proposed for *Adalia* and *Coccinella* on the basis of morphological characters.

Next, I constructed a matrix with the *Oryzaephilus* sequence as an outgroup and the *Cercyon* sequence plus the four coccinellid sequences as ingroups. Hennig86 (using both successive weighting and equal weights), NONA, and PIWE all recovered the same single most parsimonious topology, which showed the same pattern of relationships among the four coccinellid taxa as found in the preceding set of analyses: (*Calvia* (*Exochomus* (*Adalia Coccinella*))) (Fig. 4).

Finally, I constructed a larger matrix that included sequence data from a wide range of species of Coleoptera. *Cicindela* sp. (Coleoptera: Adephaga: Carabidae) was selected as an outgroup, and sequences for *Cercyon* sp., *Oryzaephilus surinamensis*, *Xantholinus* sp. (Coleoptera: Polyphaga: Staphylinidae) and *Copris* sp. (Coleoptera: Polyphaga: Scarabaeidae) were included in this matrix, in addition to the sequences from the four coccinellids. While the family Coccinellidae was clearly monophyletic in the single most parsimonious cladogram resulting from analyses of the resulting matrix with Hennig86 (using both successive weighting and equal weights), NONA, and PIWE, the relationships among the four species of coccinellids were again problematic: (*Calvia* (*Exochomus* (*Adalia Coccinella*))) (Fig. 4).

DISCUSSION AND CONCLUSIONS

All but one of the most parsimonious topologies reported here contradict the published neighbor-joining tree of Howland and Hewitt (1995), clearly demonstrating that these authors' results are not robust with respect to the choice of tree construction algorithm. Furthermore, the selection of different outgroup taxa in the present study results in the expression of strikingly different patterns of relationships among the four species of Coccinellidae. The results reported here suggest that the portion of the mitochondrial cytochrome oxidase I gene sequenced by Howland and Hewitt (1995) is simply too variable and too highly convergent to accurately reflect the phylogenetic history of Coccinellidae. Researchers interested in reconstructing the phylogeny of Coccinellidae will therefore either need to sequence additional portions of the mitochondrial cytochrome oxidase I gene, or find another gene to study.

ACKNOWLEDGMENTS

Support for the author was provided by a Smithsonian Institution Postdoctoral Research Fellowship, sponsored by Terry L. Erwin. Computer analyses were performed at Cornell University while the author was a student of James K. Liebherr. Assistance with computer software was provided by Kevin C. Nixon at Cornell University. Natalia J. Vandenberg of the USDA Systematic Entomology Laboratory provided helpful comments on the manuscript, and also pointed out that H. Sasaji and H. Kamiya are different names for the same coccinellid worker.

LITERATURE CITED

Farris, J. S. 1988. Hennig86: computer program for parsimony analysis, and documentation. Published by the author, Port Jefferson Station, New York.

Fürsch, H. 1996. Taxonomy of coccinellids, 1995 version. Coccinella, newsletter for systematic research in coccinellids 6:28–30.

Goloboff, P. 1993a. PIWE: computer program for weighted parsimony analysis. Published by the author, L. H. Bailey Hortorium, Cornell University, Ithaca, New York.

Goloboff, P. 1993b. NONA: computer program for parsimony analysis. Published by the author, L. H. Bailey Hortorium, Cornell University, Ithaca, New York.

Gordon, R. D. 1985. The Coccinellidae (Coleoptera) of American north of Mexico. Journal of the New York Entomological Society 93:1–912.

Howland, D. E. and G. M. Hewitt. 1995. Phylogeny of the Coleoptera based on mitochondrial cytochrome oxidase I sequence data. Insect Molecular Biology 4(3):203–215.

Kamiya, H. 1965. Comparative morphology of larvae of the Japanese Coccinellidae, with special reference to the tribal phylogeny of the family. Memoirs of the Faculty of Liberal Arts, Fukui University (Series II, Natural Science) 14(5):83–100.

Klausnitzer, B. 1970. Zur Larvalsystematik der mitteleuropäischen Coccinellidae (Coleoptera). Entomologische Abhandlungen (Dresden) 38:55–110.

Kovář, I. 1996. Phylogeny. Pp. 19–31 *in* I. Hodek and A. Honěk, eds. Ecology of Coccinellidae. Kluwer Academic Publishers, Dordrecht.

Lawrence, J. F. and A. F. Newton, Jr. 1995. Families and subfamilies of Coleoptera (with selected genera, notes, references, and data on family-group names). Pp. 780–1006 *in* J. Pakaluk and S. A. Ślipiński, eds. Biology, Phylogeny, and Classification of Coleoptera: Papers celebrating the 80th birthday of Roy A. Crowson. Muzeum i Instytut Zoologii PAN, Warszawa.

Nixon, K. C. 1999. WINCLADA: computer program for cladistic data analysis, BETA version. Published by the author, L. H. Bailey Hortorium, Cornell University, Ithaca, New York.

Phuoc, D. T. and F. W. Stehr. 1974. Morphology and taxonomy of the known pupae of Coccinellidae (Coleoptera) of North America, with a discussion of phylogenetic relationships. Contributions of the American Entomological Institute 10(6):i-v + 1–125.

Sasaji, H. 1968. Phylogeny of the family Coccinellidae (Insecta: Coleoptera). Etizenia 35:1–37 + pls. 1–8.

Received 21 February 2001; accepted 8 May 2001.